

Virulence Gene Typing of Methicillin-susceptible *Staphylococcus aureus* (MSSA) Isolates in Universiti Kebangsaan Malaysia Medical Centre (UKMMC)

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ABSTRACT

We determined the presence of four virulence genes: (*cna*, *seh*, PVL, TSST-1) using multiplex PCR in 880 isolates of methicillin-susceptible *Staphylococcus aureus* (MSSA) collected from the Universiti Kebangsaan Malaysia Medical Centre (UKMMC) in 2009. We found that 51.59% (454/880) of the strains had *cna*; 21.82% (192/880) possessed *seh*; 10.23% (90/880) had PVL and 6.82% (60/880) harboured TSST-1. Although methicillin-susceptible, MSSA carries important virulence genes which could affect patient's clinical course.

INTRODUCTION

Staphylococcus aureus is a major nosocomial pathogen that causes a wide range of diseases including endocarditis, osteomyelitis, pneumonia, toxic-shock syndrome, food poisoning, carbuncle and boils [1]. Its pathological importance makes it a longstanding subject of epidemiological investigation. In recent years, such studies have typically involved genotyping via a variety of molecular techniques. In one such effort, researchers have used the presence or absence of virulence elements to characterize isolates [2], where a multiplex-PCR protocol was developed for virulence gene detection of *S. aureus* [3]. Epidemiological studies are usually carried out for methicillin-resistant *S. aureus* (MRSA) due to its importance in drug resistance. In comparison, less emphasis has been placed on methicillin-susceptible *S. aureus* (MSSA) isolates, even though they are often more genetically variable and have a higher hospital prevalence (20-40%) than MRSA (5-10%) [4,5,6]. In this pilot study on MSSA isolates in our university hospital, we collected the index (first isolate from each patient) MSSA isolates of all MSSA infections reported in the hospital in 2009 and detected the presence of four clinically important virulence genes in these isolates.

MATERIALS AND METHODS

BACTERIAL STRAINS

The Universiti Kebangsaan Malaysia Medical Centre (UKMMC) is a teaching hospital with a capacity of 900

beds located in the centre of Kuala Lumpur. From January to December 2009, a total of 880 cases of MSSA related infections from various wards of the hospital were reported. We collected the index MSSA isolate of each case and established these as strains. Most strains were isolated from tissue and wound swabs. Species identification of staphylococci was performed by the Department of Medical Microbiology and Immunology, UKMMC. Strains were stocked from time of isolation in Brain Heart Infusion broth with 40% glycerol at -70°C.

GENOMIC DNA ISOLATION

Chromosomal DNA of each MSSA strain was extracted using DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instruction. The quantity and quality of DNA were determined spectrophotometrically. Extracted DNA samples were stored at -20°C.

VIRULENCE GENE TYPING

The presence of four virulence genes, *cna*, *seh*, PVL and TSST-1 in our collection of MSSA strains were determined by a modified multiplex-PCR (M-PCR) protocol [7]. M-PCR was performed in 25 µl reaction volumes containing the following: 2x PCR buffer (PROMEGA), 3 mM MgCl₂, 400 µM of each dNTP, 2.5 U *Taq* DNA Polymerase, 1 µl DNA template and 10 mM of each primer. Primer sequences for the genes are shown in Table 1. The cycle program for M-PCR was as follows: 1 cycle of pre-denaturation for 4 min at 94°C; 30 cycles of 94°C for 2 min (denaturation), 58°C for 1 min (annealing) and 72°C for 2 min (extension); and a final

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Table 1. Primers used for detection of virulence genes

Primer	Sequence	Product size (bp)	Reference
<i>cna</i> -1	5'-ACACCAGACGGTGCAACAATTA-3'	815	7
<i>cna</i> -2	5'-AGCAATACCGTTTGCATCTGTTA-3'		
<i>seh</i> -F	5'-ATTACATCATATGCGAAAGCAG 3'	555	7
<i>seh</i> -R	5'-ATGTCGAATGAGTAATCTCTAG-3'		
<i>pvl</i> -F	5'-ATGTCTGGACATGATCCAA-3'	970	7
<i>pvl</i> -R	5'-AACTATCTCTGCCATATGGT-3'		
TSST-1	5'-TGATATGTGGATCCGTCAT-3'	387	7
TSST-1	5'-AAACACAGATGGCAGCAT-3'		

extension at 72°C for 5 min. M-PCR products were analysed by gel electrophoresis with a 1.5% agarose gel (Sigma). *S. aureus* strains 81/108 (PVL), WIS (TSST) and clinical isolate MSSA 251 (*cna*, *seh*) were used as positive controls for M-PCR. 81/108 and WIS were kindly provided by Keiichi Hiramatsu and Teruyo Ito (Juntendo University, Japan).

RESULTS AND DISCUSSION

More attention has been focused towards MRSA as they are usually multidrug resistant and pose a challenge for antibiotic prescription [8]. Nevertheless, MSSA has a higher prevalence than MRSA in most hospitals, and they were reported to bring certain degree of morbidity and mortality if not properly monitored [9]. Even though they are usually susceptible to many antibiotics, MSSA has been reported to harbor various virulence genes, such as PVL, which could cause potentially fatal necrotizing pneumonia [2].

Our university hospital is a tertiary hospital which not only accepts admission from patients residing in Kuala Lumpur, but also referrals from various hospitals in the rural areas. In 2009, the percentage of MSSA infections

among *S. aureus* cases in UKMMC was as high as 73.4% (880 cases out of 1199 *S. aureus* infections). This is almost similar to another tertiary hospital in Spain (70.4%) [10]. In contrast, MSSA prevalence in Australian and Nepalese tertiary hospitals were lower at 62.7 and 55.1%, respectively [8, 9].

In this study, we determined the prevalence of four virulence genes, *cna*, *seh*, PVL and TSST-1 in index MSSA isolates collected from various wards of UKMMC in 2009. *cna* is a staphylococcal virulence factor involved in attachment of *S. aureus* to cells or extracellular matrices, where it codes for a collagen adhesin [11]. Collagen adhesin is an important step for the pathogenesis of staphylococcal arthritis and osteomyelitis; it mediates the attachment of *S. aureus* cells to cartilage [12]. We found 51.59% of the MSSA strains in our study harboured the *cna* gene, which was also the most prevalent virulence factor among the four genes that we investigated (Figure 1). About 12% (105/880) of the MSSA cases in UKMMC in 2009 were from the orthopedic ward (unpublished observation), where *cna* was detected in 55.24% (58/105) of these strains.

seh encodes for staphylococcal enterotoxin H (SEH), which is one of the newly reported staphylococcal

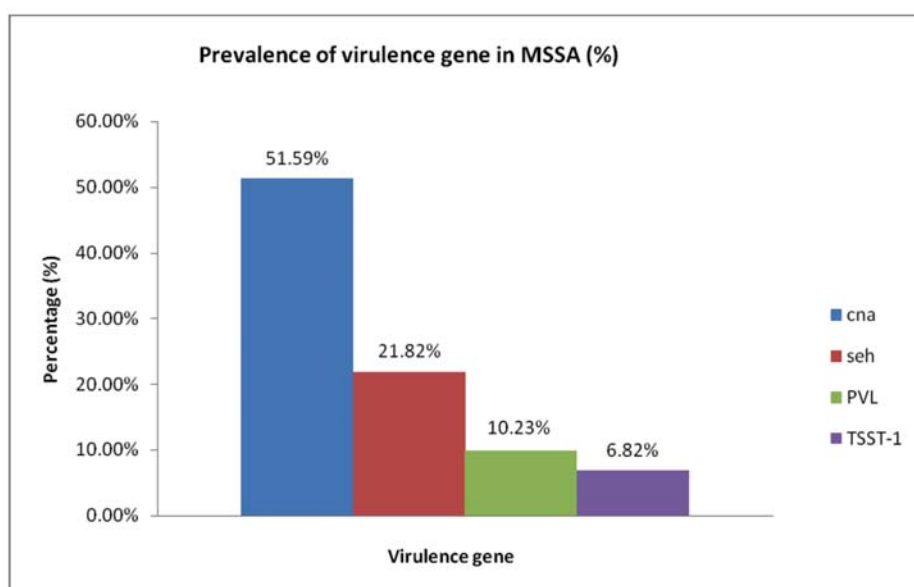


Figure 1. The prevalence of 4 virulence genes (*cna*, *seh*, PVL and TSST-1) in index MSSA strains isolated from UKMMC, 2009

enterotoxin [13]. Reports show that most *seh*-harboring *S. aureus* isolates were able to produce a significant amount of SEH, leading to food poisoning and staphylococcal superantigen-related diseases. In UKMMC, the prevalence of the *seh* gene was 21.82%, which is higher than those reported by Becker et al. (5%) [14] and Peacock et al. (15%) [15]. These differences in prevalence might be due to the role of *seh* in invasive illnesses [16]: 50% of the strains used in the studies of Becker et al. and Peacock et al. were isolated from the anterior nares of healthy individuals, therefore the prevalence of *seh* in their strains were lower compared to our study, where all of our MSSA were isolated from ill, hospitalized patients.

Interestingly, the prophage sequence encoding Panton-Valentine leukocidin (PVL), a cytotoxin usually associated with community-acquired MRSA, was detected in 10.23% of MSSA strains and this prevalence was higher than another report by Sila et al. (3%) [2]. PVL lyses leukocytes and several findings linked PVL positive strains to necrotizing pneumonia and severe skin infections [17]. Nevertheless, it is still controversial if the cytotoxin itself is pathogenic [17].

Toxic shock syndrome (TSS) is an acute systemic disease characterised by fever, hypotension, myalgia, rash, multiple-organ failure, and late desquamation of the hands and feet [18]. It is caused by the Toxic Shock Syndrome Toxin (TSST), a superantigen produced by *S. aureus* isolates carrying the *tst* gene, where TSST-1 is the common form of exotoxin related to TSS. In our study, the TSST-1 gene was present in 6.82% isolates, which is comparable to that of Sila et al's [2] investigation. Nevertheless, it is lower than those published by Becker et al. [14] who reported an 18% and 22% prevalence of TSST-1 in strains isolated from blood and nasal specimens, respectively.

CONCLUSION

Most *S. aureus* isolates from UKMMC in 2009 were still susceptible to methicillin (73.4%). Nevertheless, this current study on the prevalence of four important virulence genes in these isolates revealed that about half (58.75%) of these MSSA isolates harboured virulence factors, where the most commonly detected virulence gene was *cna* (51.59%). Even though MSSA infections are generally easier to manage as they are commonly susceptible to most available antibiotics, infections with MSSA should be treated with caution as they could still serve as reservoirs of virulence factors and introduce complications into patients' clinical course.

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